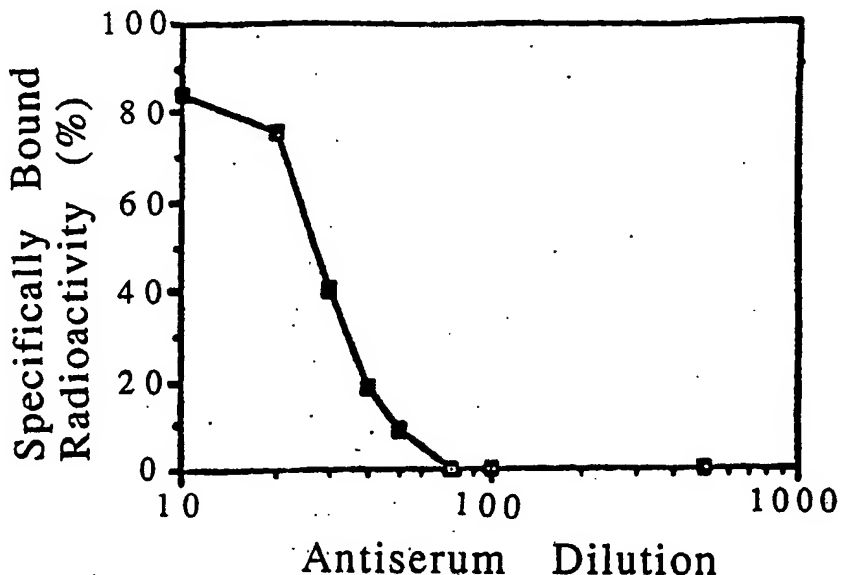# PCT

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification [5] : C07K 15/06, C12P 21/08 A61K 39/395, C07K 7/08, 7/10	A1	(11) International Publication Number: WO 91/09059
		(43) International Publication Date: 27 June 1991 (27.06.91)

(54) Title: ANTIBODY ANTAGONISTS OF HUMAN INTERLEUKIN-4

(57) Abstract

Two kinds of antibody antagonists of the binding of human IL-4 to cellular receptors are provided by this invention. Some of the antagonists bind to specific regions of IL-4 which are believed to be involved in interactions between IL-4 and its receptors. Because of this specific binding by the antibodies to the IL-4, the binding of the IL-4 to the receptors is substantially inhibited. The other antibody antagonists of the invention are anti-idiotypic antibodies which, while lacking IL-4 activity, appear to mimic IL-4 and to compete directly with it for binding to the cellular receptors. Polypeptides used to make the antibody antagonists are also provided, together with methods for using the antagonists to inhibit the binding of IL-4 to its cellular receptors.

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ANTIBODY ANTAGONISTS OF HUMAN INTERLEUKIN-4

10

BACKGROUND OF THE INVENTION

Interleukin-4 (IL-4) is a protein which affects a broad
15   spectrum of hematopoietic cells [Strober et al., Pediatr. Res. 24:549
(1988)]. IL-4 enhances a number of activities including macrophage
function, IgG and IgE production, and the proliferation of
immunoglobulin-stimulated B cells, antigen-stimulated T cells and
erythropoietin-stimulated red blood cell progenitors. It also increases
20   the proliferation of IL-3-stimulated mast cells.

Together with IgE, mast cells play a central role in allergic
reactions. Mast cells are granule-containing connective tissue cells
which are located proximally to capillaries throughout the body, with
25   especially high concentrations in the lungs, skin and gastrointestinal
and genitourinary tracts. Following exposure to an antigenic substance,
mast cells degranulate and release chemical mediators such as
histamine, serotonin, heparin, prostaglandins etc. to produce an allergic
reaction.
30

Because of the stimulatory effects of IL-4 on IgE production
and mast cell proliferation, an antagonist of IL-4 may be useful for the
treatment of allergies by decreasing mast cell growth and IgE
production.
35

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Some investigators have used antibodies to antagonize
the biological activity of IL-4. For example, Finkelman et al. [Proc. Natl.
Acad. Sci. USA 83:9675 (1986)] used a monoclonal antibody against
BSF-1 (now called IL-4) to inhibit IL-4-induced production of IgE in mice
5   infected with the nematode parasite Nippostrongylus brasiliensis or
injected with a purified goat antibody to mouse IgD. Both treatments
were known to stimulate IgE production; the latter treatment was also
known to stimulate IL-4 secretion.

10   More recently, Chretien et al. [J. Immunol. Meth. 117:67
(1989)] reported that polyclonal rabbit antiserum to partially purified
recombinant human IL-4 neutralized some of the biological activities of
IL-4 in vitro. Monoclonal antibodies against synthetic polypeptides
having amino acid sequences corresponding to residues 3-18, 31-46,
15   52-65 and 112-127 of mature human IL-4, however, failed to neutralize
the bioactivity of IL-4 although they bound to the protein.

SUMMARY OF THE INVENTION

20   This invention provides polypeptides containing from about
5 to about 26 amino acid residues which have amino acid sequences
corresponding to the sequence of amino acid residues 61 to 82 or 104
to 129 of human IL-4, or a subsequence thereof. Preferred polypeptides
have the amino acid sequences

25

Lys-Asp-Thr-Arg-Cys,

Thr-Ala-Gln-Gln-Phe-His-Arg-His,

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Lys-Asp-Thr-Arg-Cys-Leu-Gly-Ala-Thr-Ala-
Gln-Gln-Phe-His-Arg-His-Lys-Gln-Leu-Ile-
Arg-Phe and

5   Ala-Asn-Gln-Ser-Thr-Leu-Glu-Asn-Phe-Leu-
Glu-Arg-Leu-Lys-Thr-Ile-Met-Arg-Glu-Lys-
Tyr-Ser-Lys-Cys-Ser-Ser.

The present invention further provides antibodies which
10   inhibit the binding of human IL-4 to cellular receptors and specifically
bind to such IL-4 and to polypeptides containing from about 5 to about
26 amino acid residues and having amino acid sequences
corresponding to the sequence of amino acid residues 61 to 82 or 104
to 129 of human IL-4, or a subsequence thereof, which antibodies inhibit
15   the binding of human IL-4 to cellular receptors.

This invention still further provides methods for making
antibodies which specifically bind to and inhibit the binding of human
IL-4 to cellular receptors, comprising administering to an animal a
20   sufficient quantity of a polypeptide containing from about 5 to about 26
amino acid residues and having an amino acid sequence
corresponding to the sequence of amino acid residues 61 to 82 or 104
to 129 of human IL-4, or a subsequence thereof, whereby the animal
produces antibodies against the polypeptide which specifically bind to
25   human IL-4 and inhibit the binding of human IL-4 to cellular receptors.

This invention still further provides anti-idiotypic antibodies
against the above-mentioned antibodies. These antibodies presumably
antagonize the biological activity of IL-4 by competing with IL-4 for
30   binding to its cellular receptors.

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This invention still further provides a method for inhibiting the binding of human IL-4 to cellular receptors, comprising contacting human IL-4 with an antibody which specifically binds to human IL-4 and to a polypeptide containing from about 5 to about 26 amino acid

5   residues and having an amino acid sequence corresponding to the sequence of amino acid residues 61 to 82 or 104 to 129 of human IL-4, or a subsequence thereof, which antibody inhibits the binding of human IL-4 to cellular receptors.

10   This invention still further provides a method for inhibiting the binding of human IL-4 to cellular receptors, comprising contacting cells bearing receptors for human IL-4 with anti-idiotypic antibodies against an antibody which specifically binds to human IL-4 and to a polypeptide containing from about 5 to about 26 amino acid residues

15   and having an amino acid sequence corresponding to the sequence of amino acid residues 61 to 82 or 104 to 129 of human IL-4, or a subsequence thereof, which anti-idiotypic antibodies inhibit the binding of human IL-4 to cellular receptors.

20   The antibody antagonists of the invention are useful in in vitro receptor binding studies to determine the mechanism of action of IL-4 and/or to identify agonists or other antagonists of IL-4. As noted above, they may also be useful for the treatment of allergies by decreasing IL-4-stimulated mast cell proliferation and IgE production.

25

BRIEF DESCRIPTION OF THE FIGURES

This invention can be more readily understood by reference to the accompanying figures, in which:

30

Fig. 1 shows the amino acid sequence of mature human IL4, from the amino- to the carboxyl-terminus.

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Fig. 2 is a graphical representation of the binding of IL-4 (lower curve) and polypeptide No. 7 (upper curve; see Table 1) by a rabbit IgG fraction against the polypeptide, in direct ELISA analyses. The amount of protein/ polypeptide bound in picomoles is shown as a 5   function of absorbance at 414 nm.

Fig. 3 is a graphical representation of the inhibition of the specific binding of ^{125}I-IL-4 to Daudi cells by a rabbit IgG fraction against polypeptide No. 7, showing percent specifically bound 10   radioactivity as a function of increasing IgG concentration.

Fig. 4 is a graphical representation of the inhibition of the specific binding of [125] I-IL-4 to Daudi cells by anti-idiotypic antiserum 1448, showing % inhibition of specifically bound radioactivity as a 15   function of decreasing antiserum concentration.

Fig. 5 is a graphical representation of the results of epitope analysis performed on rabbit antiserum against polypeptide No. 7. ELISA absorbance produced by binding of the antiserum to a series of 20   octapeptides used in the analysis is shown. The numbers of the octapeptides correspond to the numbers in Table 3.

Fig. 6 is a graphical representation of the results of epitope analysis performed on rabbit antiserum against polypeptide No. 6. 25   ELISA absorbance produced by binding of the antiserum to a series of octapeptides used in the analysis is shown. The antiserum used to obtain the results shown in panel A was collected early in the course of immunization of the rabbit and did not inhibit the binding of ^{125}I-IL-4 to Daudi cells. The antiserum used in panel B was collected later and was 30   a strong inhibitor of the binding of the labeled IL-4. The numbers of the octapeptides correspond to the numbers in Table 4.

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DESCRIPTION OF THE INVENTION

All references cited herein are hereby incorporated in their entirely by reference. Amino acid sequences of polypeptides shown are
5   in the standard one-letter or three-letter form (Lehninger, Principles of Biochemistry, 1982, Worth Publishers Inc., New York, p. 96).

The present invention provides antibodies which antagonize the binding of human IL-4 to cellular receptors by (a)
10 combining with a region of the IL-4 which apparently is involved in interactions with the receptors or by (b) mimicing IL-4 itself, thereby competing with it for binding to the cellular receptors. Because IL-4 stimulates the production of IgE antibodies and the proliferation of mast cells, two effectors of allergic responses, the antibody antagonists of the
15 invention are useful in the treatment of allergies. They also are useful in in vitro IL-4 receptor binding systems, to elucidate the mechanism of action of IL-4 or to screen for other IL-4 antagonists or agonists.

As used herein, human "IL-4" means a protein which (a)
20 has an amino acid sequence that is substantially identical to the sequence of mature, human IL-4 shown in Fig. 1 and (b) has biological activity that is common to native IL-4.

Substantial identity of amino acid sequences means that
25 the sequence of another IL-4 compared to the sequence of Fig. 1 is identical or differs by one or more amino acid alterations (deletions, additions, substitutions) that do not substantially impair biological activity.

30 Of course, the amino acid sequences in the IL-4 regions mentioned above may differ in the case of substantially identical IL-4s.

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Investigations with synthetic polypeptides described below
have shown that there are two regions within the human IL-4 molecule
which appear to be involved in receptor binding. For convenient
reference, the amino acid sequences of these polypeptides will be
5   defined herein by the positions of the residues in the amino acid
sequence of mature human IL-4 shown in Fig. 1, with 1 being the amino-
terminal histidine residue and 129 being the carboxyl-terminal serine
residue.

10   As a result of these investigations, it has been found that
synthetic polypeptides having amino acid sequences corresponding to
the sequences of residues 52 to 82 and 104 to 129 or subsequences
thereof of human IL-4 can be used as antigens to elicit the production in
animals of antibodies which can bind to the polypeptides and to human
15   IL-4. Because of their ability to bind to such specific regions of IL-4, the
antibodies of the invention inhibit at least 60% of the specific binding of
^{125}I-IL-4 to cells bearing receptors for IL-4.

The largest of the foregoing binding regions of IL-4
20   (residues 52-82) contains about 30 amino acid residues. It is well
known in the art that antigenic determinants (epitopes) generally contain
at least about 5 amino acid residues [Ohno et al., Proc. Natl. Acad. Sci.
USA 82:2945 (1985)]. Therefore, the polypeptides of the invention
comprise from about 5 to about 30 amino acid residues and have the
25   above-mentioned amino acid sequences. Whether a given polypeptide
falls within the scope of this invention can readily be determined by
routine experimentation using the methods described below.

The polypeptides are synthesized by a suitable method
30   such as by exclusive solid phase synthesis, partial solid phase methods,
fragment condensation or classical solution synthesis. The polypeptides
are preferably prepared by solid phase peptide synthesis as described
by Merrifield, J. Am. Chem. Soc. 85:2149 (1963). The synthesis is
carried out with amino acids that are protected at the alpha-amino

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terminus. Trifunctional amino acids with labile side-chains are also
protected with suitable groups to prevent undesired chemical reactions
from occurring during the assembly of the polypeptides. The alpha-
amino protecting group is selectively removed to allow subsequent
5 reaction to take place at the amino-terminus. The conditions for the
removal of the alpha-amino protecting group do not remove the side-
chain protecting groups.

The alpha-amino protecting groups are those known to
10 be useful in the art of stepwise polypeptide synthesis. Included are
acyl type protecting groups (e.g., formyl, trifluoroacetyl, acetyl),
aromatic urethane type protecting groups [e.g., benzyloxycarbonyl
(Cbz), substituted benzyloxycarbonyl and 9-fluorenylmethyloxy-
carbonyl (Fmoc)], aliphatic urethane protecting groups [e.g., t-
15 butyloxycarbonyl (Boc), isopropyloxycarbonyl,
cyclohexyloxycarbonyl] and alkyl type protecting groups (e.g., benzyl,
triphenylmethyl). The preferred protecting group is Boc. The side-
chain protecting groups for Tyr include tetrahydropyranyl, tert.-butyl,
trityl, benzyl, Cbz, 4-Br-Cbz and 2,6-dichlorobenzyl. The preferred
20 side-chain protecting group for Tyr is 2,6-dichlorobenzyl. The side-
chain protecting groups for Asp include benzyl, 2,6-dichlorobenzyl,
methyl, ethyl and cyclohexyl. The preferred side-chain protecting
group for Asp is cyclohexyl. The side-chain protecting groups for Thr
and Ser include acetyl, benzoyl, trityl, tetrahydropyranyl, benzyl, 2,6-
25 dichlorobenzyl and Cbz. The preferred protecting group for Thr and
Ser is benzyl. The side-chain protecting groups for Arg include nitro,
Tos, Cbz, adamantyloxycarbonyl and Boc. The preferred protecting
group for Arg is Tos. The side-chain amino group of Lys may be
protected with Cbz, 2-Cl-Cbz, Tos or Boc. The 2-Cl-Cbz group is the
30 preferred protecting group for Lys.

The side-chain protecting groups selected must remain
intact during coupling and not be removed during the deprotection of
the amino-terminus protecting group or during coupling conditions.

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The side-chain protecting groups must also be removable upon the completion of synthesis, using reaction conditions that will not alter the finished polypeptide.

5.   Solid phase synthesis is usually carried out from the carboxyl-terminus by coupling the alpha-amino protected (side-chain protected) amino acid to a suitable solid support. An ester linkage is formed when the attachment is made to a chloromethyl or hydroxymethyl resin, and the resulting polypeptide will have a free

10   carboxyl group at the C-terminus. Alternatively, when a benzhydrylamine or p-methylbenzhydrylamine resin is used, an amide bond is formed and the resulting polypeptide will have a carboxamide group at the C-terminus. These resins are commercially available, and their preparation has described by

15   Stewart et al., "Solid Phase Peptide Synthesis" (2nd Edition), Pierce Chemical Co., Rockford, IL., 1984.

The C-terminal amino acid, protected at the side-chain if necessary and at the alpha-amino group, is coupled to the

20   benzhydrylamine resin using various activating agents including dicyclohexylcarbodiimide (DCC), N,N'-diisopropylcarbodiimide and carbonyldiimidazole. Following the attachment to the resin support, the alpha-amino protecting group is removed using trifluoroacetic acid (TFA) or HCl in dioxane at a temperature between 0° and 25°C.

25   Dimethylsulfide is added to the TFA after the introduction of methionine (Met) to suppress possible S-alkylation. After removal of the alpha-amino protecting group, the remaining protected amino acids are coupled stepwise in the required order to obtain the desired sequence.

30

Various activating agents can be used for the coupling reactions including DCC, N,N'-diisopropylcarbodiimide, benzotriazol-1-yl-oxy-tris-(dimethylamino)-phosphonium hexafluorophosphate (BOP) and DCC-hydroxybenzotriazole (HOBt).

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Each protected amino acid is used in excess (>2.0 equivalents), and
the couplings are usually carried out in N-methylpyrrolidone (NMP)
or in DMF, CH_2Cl_2 or mixtures thereof. The extent of completion of
the coupling reaction is monitored at each stage, e.g., by the
5   ninhydrin reaction as described by Kaiser et al., Anal. Biochem.
34:595 (1970). In cases where incomplete coupling is found, the
coupling reaction is repeated. The coupling reactions can be
performed automatically with commercially available instruments.

10   After the entire assembly of the desired polypeptide, the
polypeptide-resin is cleaved with a reagent such as liquid HF for 1-2
hours at 0°C, which cleaves the polypeptide from the resin and
removes all side-chain protecting groups. A scavenger such as
anisole is usually used with the liquid HF to prevent cations formed
15   during the cleavage from alkylating the amino acid residues present
in the polypeptide. The polypeptide-resin may be deprotected with
TFA/dithioethane prior to cleavage if desired.

          Side-chain to side-chain cyclization on the solid
20   support requires the use of an orthogonal protection scheme which
enables selective cleavage of the side-chain functions of acidic
amino acids (e.g., Asp) and the basic amino acids (e.g., Lys). The 9-
fluorenylmethyl (Fm) protecting group for the side-chain of Asp and
the 9-fluorenylmethyloxycarbonyl (Fmoc) protecting group for the
25   side-chain of Lys can be used for this purpose. In these cases, the
side-chain protecting groups of the Boc-protected polypeptide-resin
are selectively removed with piperidine in DMF. Cyclization is
achieved on the solid support using various activating agents
including DCC, DCC/HOBt or BOP. The HF reaction is carried out on
30   the cyclized polypeptide-resin as described above.

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Recombinant DNA methodology can also be used to prepare the polypeptides. The known genetic code, tailored if desired with known preferred codons for more efficient expression in a given host organism, can be used to synthesize oligonucleotides

5   encoding the desired amino acid sequences. The phosphoramidite solid support method of Matteucci et al. [J. Am. Chem. Soc. 103:3185 (1981)] or other known methods can be used for such syntheses. The resulting oligonucleotides can be inserted into an appropriate vector and expressed in a compatible host organism.

10

The polypeptides of the invention can be purified using HPLC, gel filtration, ion exchange and partition chromatography, countercurrent distribution or other well known methods.

15   Antibodies can be prepared against the polypeptides of the invention using standard methods. As used herein, the word "antibody" refers to both polyclonal and monoclonal antibodies. It also includes whole immunoglobulins and antigen binding fragments thereof.

20   The polyclonal antibodies can be produced by immunizing a host animal such as a rabbit, rat, goat, sheep, mouse, etc. with one of the polypeptides. Preferably, one or more booster injections are given after the initial injection, to increase the antibody titer. Blood is then drawn from the animal and serum is prepared and screened by standard

25   methods such as enzyme-linked immunosorbent assay (ELISA) using the polypeptide as the antigen.

Preferably, the immunogenicity of the polypeptides is increased by combination with an adjuvant and/or by conversion to a

30   larger form prior to immunization.

Suitable adjuvants for the vaccination of animals include but are not limited to Adjuvant 65 (containing peanut oil, mannide monooleate and aluminum monostearate); Freund's complete or

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incomplete adjuvant; mineral gels such as aluminum hydroxide,
aluminum phosphate and alum; surfactants such as hexadecylamine,
octadecylamine, lysolecithin, dimethyldioctadecylammonium bromide,
N,N-dioctadecyl-N',N'-bis(2-hydroxymethyl) propanediamine,
5   methoxyhexadecylglycerol and pluronic polyols; polyanions such as
pyran, dextran sulfate, poly IC, polyacrylic acid and carbopol; peptides
such as muramyl dipeptide, dimethylglycine and tuftsin; and oil
emulsions. The polypeptides could also be administered following
incorporation into liposomes or other microcarriers.
10

         The immunogenicity of the polypeptides can also be
enhanced by cross-linking or by coupling to an immunogenic carrier
molecule (i.e., a macromolecule having the property of independently
eliciting an immunological response in a host animal, to which the
15   polypeptides of the invention can be covalently linked). Cross-linking or
conjugation to a carrier molecule may be required because small
polypeptides sometimes act as haptens (molecules which are capable
of specifically binding to an antibody but incapable of eliciting antibody
production, i.e., they are not immunogenic). Conjugation of such
20   polypeptides to an immunogenic carrier molecule renders the fragments
immunogenic through what is commonly known as the "carrier effect".

         Suitable carrier molecules include, e.g., proteins and
natural or synthetic polymeric compounds such as polypeptides,
25   polysaccharides, lipopolysaccharides etc. A useful carrier is a glycoside
called Quil A. which has been described by Morein et al., Nature
308:457 (1984). Protein carrier molecules are especially preferred,
including but not limited to keyhole limpet hemocyanin and mammalian
serum proteins such as human or bovine gammaglobulin, human,
30   bovine or rabbit serum albumin, or methylated or other derivatives of
such proteins. Other protein carriers will be apparent to those skilled in
the art. Preferably, but not necessarily, the protein carrier will be foreign
to the host animal in which antibodies against the polypeptides are to be
elicited.

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Covalent coupling to the carrier molecule can be carried out using methods well known in the art, the exact choice of which will be dictated by the nature of the carrier molecule used. When the immunogenic carrier molecule is a protein, the polypeptides of the

5   invention can be coupled, e.g., using water soluble carbodiimides such as dicyclohexylcarbodiimide or glutaraldehyde.

Coupling agents such as these can also be used to cross-link the polypeptides to themselves without the use of a separate carrier

10   molecule. Such cross-linking into aggregates can also increase immunogenicity.

Serum produced from animals thus immunized can be used directly. Alternatively, the IgG fraction can be separated from the

15   serum using standard methods such as plasmaphoresis or adsorption chromatography using IgG specific adsorbents such as immobilized Protein A.

Monoclonal antibodies can be prepared using standard

20   methods, e.g., as described by Kohler et al. [Nature 256:495 (1975); Eur. J. Immunol. 6:511 (1976)]. Essentially, an animal is immunized as described above to produce antibody-secreting somatic cells. These cells are then removed from the immunized animal for fusion to myeloma cells.

25

Somatic cells with the potential to produce antibodies, particularly B cells, are suitable for fusion with a myeloma cell line. These somatic cells may be derived from the lymph nodes, spleens and peripheral blood of primed animals. In the exemplary embodiment of this

30   invention mouse spleen cells are used, in part because these cells produce a relatively high percentage of stable fusions with mouse myeloma lines. It would be possible, however, to use rat, rabbit, frog or other cells instead.

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Specialized myeloma cell lines have been developed from
lymphocytic tumors for use in hyridoma-producing fusion procedures
[Kohler and Milstein, Eur. J. Immunol. 6:511 (1976); Shulman et al.,
Nature 276:269 (1978); Volk et al., J. Virol. 42:220 (1982)]. These cell
5   lines have been developed for at least three reasons. The first is to
facilitate the selection of fused hybridomas from unfused and similarly
indefinitely self-propagating myeloma cells. Usually, this is
accomplished by using myelomas with enzyme deficiencies that render
them incapable of growing in certain selective media that support the
10   growth of hybridomas. The second reason arises from the inherent
ability of lymphocytic tumor cells to produce their own antibodies. The
purpose of using monoclonal techniques is to obtain fused hybrid cell
lines with unlimited lifespans that produce the desired single antibody
under the genetic control of the somatic cell component of the
15   hybridoma. To eliminate the production of tumor cell antibodies by the
hybridomas, myeloma cell lines incapable of producing light or heavy
immunoglobulin chains or deficient in antibody secretion mechanisms
are used. A third reason for selection of these cell lines is for their
suitability and efficiency for fusion.
20

Many myeloma cell lines may be used for the production of
fused cell hybrids, including, e.g., P3X63-Ag8, P3/NS1-Ag4-1 (NS-1),
Sp2/0-Ag14 and S194/5.XXO.Bu.1. The P3X63-Ag8 and NS-1 cell lines
have been described by Kohler and Milstein [Eur. J. Immunol. 6:511
25   (1976)]. Shulman et al. [Nature 276:269 (1978)] developed the Sp2/0-
Ag14 myeloma line. The S194/5.XXO.Bu.1 line was reported by
Trowbridge [J. Exp. Med. 148:313 (1979)].

Methods for generating hybrids of antibody-producing
30   spleen or lymph node cells and myeloma cells usually involve mixing
somatic cells with myeloma cells in a 10:1 proportion (although the
proportion may vary from about 20:1 to about 1:1), respectively, in the
presence of an agent or agents (chemical, viral or electrical) that
promotes the fusion of cell membranes. Fusion methods have been

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described by Kohler and Milstein, supra, Gefter et al. [Somatic Cell
Genet. 3:231 (1977)], and Volk et al. (J. Virol. 42:220 (1982)]. The fusion-
promoting agents used by those investigators were Sendai virus and
polyethylene glycol (PEG). The fusion procedure of the example of the
5   present invention uses PEG.

Because fusion procedures produce viable hybrids at very
low frequency (e.g., when spleens are used as a source of somatic cells,
only one hybrid is obtained for roughly every 1 x 10^5 spleen cells), it is
10   essential to have a means of selecting the fused cell hybrids from the
remaining unfused cells, particularly the unfused myeloma cells. A
means of detecting the desired antibody-producing hybridomas among
other resulting fused cell hybrids is also necessary.

15   Generally, the selection of fused cell hybrids is
accomplished by culturing the cells in media that support the growth of
hybridomas but prevent the growth of the unfused myeloma cells, which
normally would go on dividing indefinitely. The somatic cells used in the
fusion do not maintain long-term viability in in vitro culture and hence do
20   not pose a problem. In the example of the present invention, myeloma
cells lacking hypoxanthine phosphoribosyl transferase (HPRT-negative)
were used. Selection against these cells is made in
hypoxanthine/aminopterin/thymidine (HAT) medium, a medium in which
the fused cell hybrids survive due to the HPRT-positive genotype of the
25   spleen cells. The use of myeloma cells with different genetic deficiencies
(drug sensitivities, etc.) that can be selected against in media supporting
the growth of genotypically competent hybrids is also possible.

Several weeks are required to selectively culture the fused
30   cell hybrids. Early in this time period, it is necessary to identify those
hybrids which produce the desired antibody, so that they may
subsequently be cloned and propagated. Generally, around 10% of the
hybrids obtained produce the desired antibody, although a range of from
about 1 to about 30% is not uncommon. The detection of antibody-

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producing hybrids can be achieved by any one of several standard
assay methods, including enzyme-linked immunoassay and
radioimmunoassay techniques which have been described in the
literature [see, e.g., Kennet et al. (editors), Monoclonal Antibodies and
5   Hybridomas: A New Dimension in Biological Analyses, pp. 376-384,
Plenum Press, New York (1980)].

Once the desired fused cell hybrids have been selected
and cloned into individual antibody-producing cell lines, each cell line
10   may be propagated in either of two standard ways. A suspension of the
hybridoma cells can be injected into a histocompatible animal. The
injected animal will then develop tumors that secrete the specific
monoclonal antibody produced by the fused cell hybrid. The body fluids
of the animal, such as serum or ascites fluid, can be tapped to provide
15   monoclonal antibodies in high concentration. Alternatively, the
individual cell lines may be propagated in vitro in laboratory culture
vessels. The culture medium containing high concentrations of a single
specific monoclonal antibody can be harvested by decantation, filtration
or centrifugation.
20

Whether anti-polypeptide antibodies made as described
above are suitable for use in this invention is determined by a two-part
screening procedure involving (a) ELISA analysis using the immunizing
polypeptide and human IL-4 as antigens and (b) radioligand receptor
25   binding analysis, in which inhibition of the specific binding of ^{125}I-IL-4 to
cellular receptors is measured.

Recombinant human IL-4 for use in such assays is an
article of commerce, available, e.g., from Genzyme Corporation, Boston,
30   MA. Alternatively, it can be produced using the known nucleotide
sequence of the IL-4 gene [Yokoto et al., Proc. Natl. Acad. Sci. USA
83:5894 (1986)] and standard recombinant DNA methods [see, e.g.,
International Patent Application Publication No. WO 87/02990;
Kimmenade et al., Eur. J. Biochem. 173:109 (1988)].

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ELISA analysis is carried out by standard methods such as the method of Chretien et al. [J. Immunol. Meth. 117:67 (1989)], using a polypeptide or IL-4 adsorbed to a microtiter plate. The presence of antibodies bound to the immobilized polypeptide or protein is detected

5 with a labeled anti-IgG second antibody. Such second antibodies are preferably labeled with an enzyme such as a peroxidase, glucose oxidase, b-galactosidase or alkaline phosphatase. Horseradish peroxidase can be detected by spectrophotometric analysis of its activity on a substrate such as pyrogallol, o-phenylenediamine or 2,2'-azino-

10 bis(3-ethyl-benzthiazoline-6-sulfonic acid).

Antibodies found to specifically bind to both the immunizing polypeptide and IL-4 are further evaluated for the ability to inhibit the specific binding of labeled IL-4 to receptors on appropriate

15 target cells. The anti-polypeptide antibodies of the invention are characterized by an ability to inhibit at least 60% of such binding.

Any cells bearing IL-4 receptors such as Jijoye, U-937, CCRF-CEM and CEM-CM3 cells can be used to carry out the binding

20 assay, but Daudi cells are convenient and readily available. Daudi cells are a well-characterized B lymphoblast cell line derived from a Burkitt lymphoma patient which can be purchased from the American Type Culture Collection under Accession No. ATCC CCL 213. ^{125}I-IL-4 for use in the assay can be prepared by labeling IL-4 with iodine-125 using,

25 e.g., the lactoperoxidase method [David et al., Biochemistry 13:1014 (1974)] or the method of Bolton et al. [Biochem. J. 133:529 (1973)]. Glycosylated recombinant human IL-4 is an article of commerce, available for purchase, e.g., from Genzyme Corporation, Boston, MA.

30 The anti-idiotypic antibodies of the invention are directed against antibodies specific for the IL-4 antigenic determinants present in the polypeptides of the invention. Such anti-idiotypic antibodies mimic or act like the original antigenic determinants (see, e.g., U.S. Patent No. 4,731,237 to Reagan et al.). Like IL-4 itself, these antibodies are

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presumed to bind specifically and directly to IL-4 receptors. The anti-idiotypic antibodies, however, do not possess the biological activity of IL-4.

5   Such anti-idiotypic antibodies are prepared by vaccinating an animal with an antibody (polyclonal or monoclonal) against a polypeptide of the invention. They may be recovered as a whole polyclonal antiserum or as an IgG fraction thereof, or as monoclonal antibodies produced by cloned hybridomas, as described above.

10
           Pharmaceutical compositions can be prepared which contain effective amounts of one or more of the antibodies of the invention and a physiologically acceptable carrier. Such carriers are well known to those skilled in the art. The antibodies can be
15   administered directly or in the form of a composition to a human patient for the treatment of allergies or other conditions mediated by IL-4. The pharmaceutical compositions are made by admixing a physiologically acceptable carrier with an effective amount of one or more of the antibodies.

20
           Determination of the proper dosage of an antibody of the invention for a particular situation is within the skill of the art. Generally, treatment is initiated with smaller dosages that are less than optimum. Thereafter, the dosage is increased by small increments until the
25   optimum effect under the circumstances is reached. For convenience, the total daily dosage may be divided and administered in portions during the day if desired.

           The amount and frequency of administration of the
30   antibodies of the invention will be regulated according to the judgment of the attending clinician, taking into account such factors as age, condition and size of the patient and severity of the symptom(s) being treated.

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EXAMPLES

Unless otherwise specified, percentages given below for solids in solid mixtures, liquids in liquids and solids in liquids are on a
5   wt/wt, vol/vol and wt/vol basis, respectively.

Protein determinations were carried out by the method of Lowry et al. [J. Biol. Chem. 193:265 (1951)] using bovine serum albumin as a standard. Bioassay of IL-4 was performed as described by
10   Mossman [J. Immunol. Methods 65:55 (1983)], measuring stimulation of cell proliferation as MTT (3-[4,5-Dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide) uptake in PHA-stimulated human peripheral blood lymphocytes. One unit of IL-4 activity is an amount of IL-4 which causes half-maximal stimulation in 2×10^5 cells in the assay.
15   One microgram of pure human IL-4 has about 20,000 units of activity in the assay.

Polypeptide Synthesis

20   A number of polypeptides were synthesized, the amino acid sequences of which, taken together, correspond to the amino acid sequence of the entire mature human IL-4 protein.

The polypeptides were synthesized using the solid-phase
25   method of Merrifield [J. Am. Chem. Soc. 85:2149 (1963)] and an Applied Biosystems Model 430A synthesizer. The t-butyloxycarbonyl amino protecting group and symmetrical anhydrides were employed. Following removal of the protecting groups, the polypeptides were cleaved from the resin with hydrogen fluoride.
30

Purification of the polypeptides was carried out by reversed-phase HPLC using a Rainin Dynamax® C-8 column developed with a gradient of acetonitrile in 0.1% trifluoroacetic acid. The eluate was monitored by ultraviolet absorbance at 214 nm. The

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identities of the purified polypeptides were confirmed by amino acid sequencing and mass spectral analysis, using standard methods.

5   The polypeptides produced, their amino acid sequences and the residues of mature human IL-4 (i.e., without a signal peptide; see Fig. 1) to which the polypeptide sequences correspond are shown in Table 1.

10

Table 1

Structures of the Synthetic Polypeptides

15

Polypeptide No.	Sequence	Corresponding IL-4 Residues
1	HKCDITLQEIIKTLNSLTEQKTLCTE	1-26
2	CDITLQEIIKTLNSLT	3-18
3	TEQKTLCTELTVTD	18-31
4	DIFAASKNTTEKETFC	31-46
5	ETFSRAATVLRQFYS*	43-57
6	LRQFYSHHEKDTRC	52-65
7	KDTRCLGATAQQFHRHKQLIRF	61-82
8	LKRLDRNLWGLAGLNSCPVK	83-102
9	AQQFHRHKQLIRFLKRLDRNLWG	70-92
10	CPVKEANQSTLEN	99-111
11	ANQSTLENFLERLKTIMREKYSKCSS	104-129
12	FLERLKTIMREKYSKC	112-127

20   * The amino acid sequence of polypeptide No. 5 corresponds to residues 43-57 of human IL-4, except that the cysteine residue at position 46 of human IL-4 has been replaced by a serine residue in the polypeptide.

25

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Hydrophilicity analysis of human IL-4 carried out by Hopp et al. [Proc. Natl. Acad. Sci. USA 78:3824 (1981)] shows that the region corresponding to polypeptide No. 7 contains both hydrophilic and hydrophobic residues which are predicted by secondary structure
5 models to possibly form an alpha helical region in IL-4.

Preparation and Characterization of Anti-Polypeptide Antibodies

Two milligrams of polypeptide No. 7 (Table 1)
10 corresponding to residues 61-82 of human IL-4 were dissolved in 0.4 ml of 0.5 M Tris-HCl, pH 6.8, and 0.1 ml of pertussis vaccine (source, strain 18334, heat killed, 20 Units/ml, 1/10,000 dilution thimersal). Freund's complete adjuvant (0.5 ml) was added, and the sample was homogenized in a syringe. New Zealand white rabbits were each
15 immunized with 1 ml of the sample by 0.1 ml (200 µg polypeptide) intradermal injections.

After a period of about four months and periodically thereafter, booster injections were given as above. Blood was
20 periodically withdrawn from the ear or femur veins of the rabbits and allowed to clot.

An IgG fraction was isolated from the serum of one of the rabbits by adsorbing the same onto a Protein A-Sepharose® column
25 (Pharmacia, Piscataway, NJ) equilibrated with 1.5 M glycine buffer, pH 8.9. Chromatography was carried out using standard methods by Forton Biochem. Co. The purified material was judged to be about 98% pure IgG by SDS polyacrylamide gel electrophoresis [Laemmli, Nature 227:680 (1970)]. This material was designated the antiserum 343-6 IgG
30 fraction.

Using similar methods, IgG fractions of antisera against the other polypeptides shown in Table 1 were also prepared.

ELISA was carried out on the isolated IgG fractions by coating 96-well microtiter plates (Becton-Dickinson) with about 0.25 µg of one of the various polypeptides in 50 µl of Tris-buffered saline (TBS; 50 mM Tris, 0.15 M NaCl, pH 7.0) for one hour at room temperature.

5   Following this incubation, the wells were washed five times with TBS containing 0.1% Tween 20 (polyoxyethylenesorbitan monolaurate).

The washed wells were blocked with 1% bovine serum albumin (BSA) in TBS for 1 hour at room temperature, washed five times

10   with TBS, blocked with 0.1% nonspecific IgG in TBS for 2 hours at room temperature, and washed five times as described above. Fifty-microliter aliquots of various dilutions of the IgG fractions in TBS were then added to the wells, and the plates were incubated at room temperature for 1 hour and then washed in the same way as before.

15

To each well was added 50 µl of TBS containing 2.5 ng of horseradish peroxidase-labeled goat anti-rabbit IgG, and the plates were incubated for 1 hour at room temperature. After washing as above, the wells were developed with hydrogen peroxide and 2,2-Azino-di-(3-

20   ethyl-benzthiazoline sulfonate).

Control wells were also developed in which one of the three assay components (i.e., antigen, antibody or labeled second antibody) was deleted. Samples were read in a Dynatech Model 650

25   spectrophotometer.

The results of such analysis carried out on the antiserum 343-6 IgG fraction using polypeptide No. 7 (Table 1) and human IL-4 as antigens are shown in Fig. 2. There, where absorbance at 414 nm as a

30   measure of antigen binding is shown as a function of the amount of polypeptide or IL-4 per well, it can be seen that the antibodies bound to both antigens. To produce these results, the antiserum 343-6 IgG fraction was diluted 1:200 prior to coating 50 µl aliquots onto the wells.

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To determine whether antibodies in the anti-polypeptide - IgG fractions, by specifically binding to human IL-4, could thereby inhibit the binding of the IL-4 to cellular receptors, radioligand binding analyses were carried out.

5

Purified recombinant human IL-4 expressed in CHO cells [Le et al., J. Biol. Chem. 263:10817 (1988)] was labeled with iodine-125 by a modification of the method of Bolton et al. [Biochem. J. 133:529 (1973)], using Bolton-Hunter reagent from DuPont-NEN, Boston, MA.

10   Briefly, 2 mCi of the Bolton-Hunter reagent were reacted with 5.0 µg of the purified IL-4 in 100 µl of 50 mM sodium phosphate buffer, pH 8.0, for 2 hours at 22°C. The reaction was quenched for 1 hour by the addition of an equal volume of 1.0 M glycine.

15   The iodinated protein was isolated by gel filtration in a PD-1 column (Pharmacia, Piscataway, NJ) equilibrated with 0.2% gelatin in 50 mM sodium phosphate, pH 7.4. Radioactive material eluting from the column in the void volume was pooled and analyzed. The specific radioactivity of the labeled IL-4 was 1500 Ci/mmole as

20   determined by the self displacement method of Calvo et al., Biochem. J. 212:259 (1983)], and the molar incorporation ratio was 0.68 mole of iodine per mole of protein.

One-tenth milliliter volumes of serial dilutions of the various

25   anti-polypeptide IgG fractions in binding medium [RPMI 1640 with 10% fetal calf serum (FCS)] were incubated with constant amounts of $125I$-IL-4 (about 2×10^5 cpm) in 1.0 ml of binding medium in 1.5 ml tubes for 18 hours at 4°C prior to performance of binding assays. Following this preincubation, the contents of the tubes were combined

30   with 2×10^6 Daudi cells, and the mixtures were incubated for 2 hours at 4°C.

Following the incubation, the cells were pelleted by centrifugation at 800 or 12,000 x g for 30 seconds at 4°C, and the

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supernatants were discarded. The cells were resuspended in 0.1 ml of
fresh binding medium without labeled IL-4 at 4°C, pelleted as above,
resuspended in 100 μl of assay medium and overlaid on 100 μl of
dibutyl phthalate and dioctyl phthalate (1:1). The cells were pelleted at

5 13,000 x g for 2 minutes, frozen in liquid nitrogen and were then counted
in a gamma counter. Nonspecific binding was determined in parallel
samples containing 1.0 mg of unlabeled human IL-4.

The results of the foregoing analyses are shown in Table 2.

10

Table 2

Analysis of Anti-Polypeptide IgG Fractions

Polypeptide Used As Antigen[a]	Antibody Reactivity With[b]		% Inhibition of ^{125}I-IL-4 Binding
	Polypeptide	IL-4	
1	+	-	0
2	+	+	0
3	+	+	2.4
4	+	+	0
5	+	+	8.7
6	+	+	76
7	+	+	78
8	+	-	7.5
9	+	+	39
10	+	+	26
11	+	+	60
12	+	+	0

a The amino acid sequences of the polypeptides and the
   corresponding regions within the human IL-4 molecule are shown in
   Table 1.

b In determining antibody reactivity, + means an absorbance at 414 mn
   > 0.05, after subtracting the absorbance of control wells.

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The data of Table 2 show that antibodies produced against
polypeptides corresponding to residues 52-65 (polypeptide No. 6), 61-
82 (polypeptide No. 7) and 104-129 (polypeptide No. 11) of human IL-4
were strong inhibitors of the ^{125}I-IL-4 to the Daudi cells.

5   These antibodies specifically bound to both the immunizing
polypeptides and to the IL-4, although pre-immune serum bound to
neither and had no effect on receptor binding.

As further shown in Table 2, the antibodies against

10   polypeptides 6 and 7 were equally potent in inhibiting the binding of the
labeled IL-4. Table 1 shows that these polypeptides share a common
KDTRC amino acid subsequence. Such combined evidence suggests
that this subsequence may constitute an important epitope and provides
support for polypeptides of the invention which may contain as few as 5

15   amino acid residues.

The binding inhibition produced by the polyclonal
antibodies against polypeptide No. 6 is particularly interesting. As noted
above, Chretien et al. found that a monoclonal antibody produced

20   against the same polypeptide did not neutralize the bioactivity of IL-4.
Subsequent epitope analysis described below, however, has shown
that that antibody is probably directed against residues toward the
amino terminus of the polypeptide, not against the Lys-Asp-Thr-Arg-Cys
subsequence at the carboxyl-terminus. Presumably, the polyclonal

25   antiserum of this example inhibited IL-4 binding because some of the
antibodies in it were directed against the epitope comprising this specific
subsequence.

The results obtained with the antiserum 343-6 IgG fraction

30   against polypeptide No. 7 are shown graphically in Fig. 3, wherein 2 x
10^6 Daudi cells were incubated with 50 pM ^{125}I-IL-4 and the indicated
antibody concentrations for 2 hours at 4°C. Specific binding in the
absence of antibody was 3,347 cpm. The strong binding inhibition
observed, coupled with the fact that the antibodies specifically bound to

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polypeptide No. 7 and to IL-4, suggests that the amino acid residues
against which the antibodies are directed may be exposed on the
surface of IL-4.

5   <u>Monoclonal Anti-polypeptide Antibodies</u>

Monoclonal antibodies were prepared essentially as
described by Kohler and Milstein [Nature <u>256</u>:495 (1975)]. All
incubations were carried out at 37°C. in a 5% CO_2 incubator.

10

Balb/c mice (Charles River) were immunologically
sensitized by administering 500 µl of 2,6,10,14-Tetramethylpentadecane
(Pristane) intraperitoneally (i.p.). About four days later, 250 µg of
polypeptide No. 7 (Table 1; corresponding to residues 61-82 of human
15   IL-4) were dissolved in 250 µl volumes of phosphate buffered saline
(PBS), 250 µl aliquots of Freund's complete adjuvant were added, and
the mixtures were homogenized and administered i.p. to each mouse.
About one month later, booster injections containing 1.25 µg of the
polypeptide in 1:1 diluted Freund's incomplete adjuvant were
20   administered i.p.

Three or four weeks later, final i.p. injections of 250 µg of
polypeptide No. 7 in PBS were administered. Periodically during the
course of immunization, test bleeds were made from the tail veins and
25   analyzed by ELISA as described above. Four days after the final
immunizations, the animals were sacrificed and their spleens were
removed.

The spleens were macerated between two slides in fresh
30   RPMI 1640 medium containing 100 µg/ml streptomycin and 100 units/ml
penicillin (RPMI pen/strep medium) and then transferred to a large tube.
After allowing debris to settle for 1 minute, cells in the upper layer of the
tube were transferred to a 5 ml tube. Four milliliters of the RPMI

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pen/strep medium were added.and the cells were suspended and then.
sedimented by centrifugation at about 300 x g for 8 minutes.

5.   A 5:1 ratio of spleen cells to NS-1 mouse myeloma cells·
(ATCC TIB 18) was prepared and washed once with the RPMI pen/strep
medium. After pelleting the cells as before and discarding the medium,
0.5 ml of PEG (2 g per liter in 75 mM HEPES buffer) having a molecular.
weight of about 1500 daltons was added dropwise over a period of 1
minute at 37°C, with gentle agitation every 20 seconds. The PEG
10   addition was repeated, first with 0.5 and then 1.0 ml of the PEG solution.

Following fusion, the cells were sedimented and washed
for 1 minute periods with 0.5, 1.0, 2.0, 4.0, 8.0, 16.0 and 32.0 ml of the
RPMI pen/strep medium. The fusion cells were sedimented as before
15   and the medium was discarded, after which about 1 x 10^5 spleen cells
from a naive mouse were added as feeder cells in RPMI pen/strep
medium containing 0.2933 mg/ml glutamine and 10% fetal calf serum
(FCS) and the cells were mixed and then sedimented as before. After
isolation from the mouse the day before, the feeder splenocytes had
20   been incubated overnight at 37°C in RPMI pen/strep medium containing
the glutamine and FCS.

The fusion and feeder cells were grown together for 7 days
in RPMI pen/strep medium containing 0.2933 mg/ml glutamine, 10%
25   FCS, 1 x 10^{-2} M hypoxanthine, 4 x 10^{-5} M aminopterin and 1.6 x 10^{-3} M
thymidine (HAT medium) in 96-well flat-bottom microtiter plates
(COSTAR), 150 µl per well. After this incubation period, the medium in
each well was replaced with HT medium (HAT medium lacking
aminopterin) and incubation was continued.
30

· After several days, ELISA was carried out on the
hybridoma supernatants as described above, except that a labeled anti-
mouse IgG antibody was used. Hybridomas in wells testing positive
were cloned by limiting dilution in HT medium.

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A total of 382 cloned hybridomas were produced in this way, all of which produced monoclonal antibodies. After screening these hybridomas by ELISA using polypeptide No. 7 as the antigen, 12 positive cell lines were identified. Of these, 10 were found positive by
5   ELISA screening against IL-4.

Ouchterlony screening of 8 of the positive clones in agar carried out by standard methods with immunoglobulin-specific antisera showed that 6 of the clones produced IgG1, one produced IgG2a and
10   one produced IgM antibodies.

Preparation of Anti-Idiotypic Antibodies

To produce anti-idiotypic antibodies, 1.5 mg of the
15   antiserum 343-6 IgG fraction described above in 0.5 ml of phosphate-buffered saline were added to 0.5 ml of Freund's complete adjuvant and mixed thoroughly to form an emulsion. The sample was injected subcutaneously into a sheep (Dorset crossbreed). Booster vaccinations were administered at several-week intervals thereafter in an identical
20   manner, except that Freund's incomplete adjuvant was used.

Occasional blood samples taken during the course of immunization were subjected to ELISA analysis using the antiserum 343-6 IgG fraction as the antigen as described above, except that
25   blocking with immunoglobulin was omitted and 5.0 ng of horseradish peroxidase-labeled donkey anti-sheep IgG was used as the second antibody. The sheep antiserum thus obtained (designated antiserum 1448) was found to specifically bind to the rabbit antiserum 343-6 IgG fraction but not to IL-4 or to polypeptide No. 7.
30

To determine whether sheep antiserum 1448 did indeed contain anti-idiotypic antibodies, a serial dilution of the antiserum was subjected to radioligand receptor binding analysis using ^{125}I-IL-4 and Daudi cells as described above, with the results shown in Fig. 4. Each

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sample in the assay contained 2 x 10^6 cells and 50 pM of ^{125}I-IL-4 (2 x 10^5 cpm). Specific binding in the absence of antiserum was 5,931 cpm.

As shown in Fig. 4, sheep antiserum 1448 was a strong
5   competitive inhibitor of the binding of the labeled IL-4 to the cells,
abolishing more than 80% of the specific binding at the lower dilutions.
In contrast, sheep pre-immune serum had no effect on the binding of the
IL-4.

10   Epitope Analysis

To determine which amino acid residues in polypeptides
Nos. 6 and 7 were critical to the production of antibodies which could
inhibit the binding of IL-4 to cellular receptors, epitope analysis was
15   carried out essentially as described by Geysen et al. [Proc. Natl. Acad.
Sci. USA 81: 3998 (1984)].

The method of Geysen et al. allows the rapid concurrent
synthesis on solid supports of hundreds of small polypeptides of
20   sufficient purity and in sufficient quantity to carry out ELISA, while the
polypeptides are still attached to the solid supports on which they were
synthesized. In principle, ELISA is carried out on such polypeptides
using antibodies which had been prepared against a larger polypeptide
or protein having an amino acid sequence which includes the
25   sequences of the small polypeptides. If the antibodies are specific for an
epitope within the larger immunogen that is encompassed by the small
synthetic polypeptides, the antibodies will bind to the polypeptides and
can be detected by ELISA.

30   Using the method of Geysen et al., supra, a series of 15
octapeptides was synthesized on polyethylene pins (Cambridge
Research Biochemicals, Inc., Valley Stream, N.Y.), the amino acid
sequences of which, in the aggregate, spanned all of the residues in

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polypeptide No. 7 (corresponding to residues 61-82 of mature human IL-4). The sequences of these octapeptides are shown in Table 3.

5

<u>Table 3</u>

Octapeptides Based Upon
<u>Residues 61-82 of Mature Human IL-4</u>

Octapeptide No.	Sequence	Corresponding IL-4 Residues	Center* Residue
1	KDTRCLGA	61-68	65
2	DTRCLGAT	62-69	66
3	TRCLGATA	63-70	67
4.	RCLGATAQ	64-71	68
5	CLGATAQQ	65-72	69
6	LGATAQQF	66-73	70
7	GATAQQFH	67-74	71
8	ATAQQFHR	68-75	72
9	TAQQFHRH	69-76	73
10	AQQFHRHK	70-77	74
11	QQFHRHKQ	71-78	75
12	QFHRHKQL	72-79	76
13	FHRHKQLI	73-80	77
14	HRHKQLIR	74-81	78
15	RHKQLIRF	75-82	79

10

* The center residues of the octapeptides were arbitrarily designated by adding four to the residue position in mature human IL-4, to which the N-terminal residue of each octapeptide corresponded.

15

In like fashion, a series of 17 pin-immobilized octapeptides which together spanned all of the residues corresponding to residues 47-70 of mature human IL-4 was prepared. The amino acid sequences of these octapeptides are shown in Table 4.

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Table 4

**Octapeptides Based Upon
Residues 47-70 of Mature Human IL-4**

Octa-peptide No.	Sequence	Corresponding IL-4 Residues	Center* Residue
1	RAATVLRQ	47-54	51
2	AATVLRQF	48-55	52
3	ATVLRQFY	49-56	53
4	TVLRQFYS	50-57	54
5	VLRQFYSH	51-58	55
6	LRQFYSHH	52-59	56
7	RQFYSHHE	53-60	57
8	QFYSHHEK	54-61	58
9	FYSHHEKD	55-62	59
10	YSHHEKDT	56-63	60
11	SHHEKDTR	57-64	61
12	HHEKDTRC	58-65	62
13	HEKDTRCL	59-66	63
14	EKDTRCLG	60-67	64
15	KDTRCLGA	61-68	65
16	DTRCLGAT	62-69	66
17	TRCLGATA	63-70	67

* The center residues of the octapeptides were arbitrarily designated by adding four to the residue position in mature human IL-4, to which the N-terminal residue of each octapeptide corresponded.

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To carry out epitope analysis on polypeptide No. 7,
antiserum designated 129-88 from a rabbit immunized with the
polypeptide as described above was subjected to ELISA, using the
polyethylene pin-immobilized octapeptides shown in Table 3 as the
5   antigen. This antiserum was found to strongly inhibit the binding of
^{125}I-IL-4 to Daudi cells, in an assay performed as described above. The
ELISA was carried out on antiserum 129-88 essentially as described
above in 96-well microtiter plates, except that the pins were used in the
wells instead of coating free antigen onto the wells. Prior to reading the
10   color development using a Titertek MCC 340 ELISA plate reader, the
pins were removed from the walls.

The results of this analysis is shown in Fig. 5, where
absorbance at 414 nm is shown for each of the octapeptides. The
15   numbers of the octapeptides shown in Fig. 5 correspond to the numbers
in Table 3. Strong binding of antibodies to octapeptides 5-12 can be
seen in Fig. 5. Referring to Table 3, it can be seen that the approximate
centers of these octapeptides corresponded to residues 69-76 of mature
human IL-4. These data, combined with the fact that antiserum 129-88
20   inhibited the binding of the labeled IL-4 to the cellular receptors,
suggests that residues 69-76 of mature human IL-4 constitute an
epitope(s), antibodies against which inhibit the binding of human IL-4 to
cellular receptors.

25   In a similar fashion, the immobilized octapeptides shown in
Table 4 were used to analyze rabbit antiserum produced against
polypeptide No. 6. This antiserum, designated 342-6, was evaluated
twice for ability to inhibit the binding of ^{125}-I-IL-4 to Daudi cells. Serum
samples prepared early in the course of immunization (early antiserum
30   342-6) did not inhibit labeled IL-4 binding; samples prepared later (late
antiserum 342-6) were strongly inhibitory. The results of these analyses
are shown in Fig. 6A and B for the early and late antiserum, respectively.
The numbers of the octapeptides shown in Fig. 6 correspond to the
numbers in Table 4.

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As shown in Fig. 6A, the non-inhibitory early antiserum 342-6 against polypeptide No. 6 contained antibodies reactive with octapeptides 3-7 and 9-13. Referring to Table 4, it can be seen that the centers of these octapeptides corresponded approximately to residues

5   53-57 and 59-63, respectively, of mature human IL-4.

The late, inhibitory antiserum 342-6 produced a similar binding pattern (Fig. 6B), except that it also contained antibodies which exhibited stronger binding to octapeptides 11-16, the centers of which

10   corresponded to residues 61-66 of the human IL-4. The data of panels A and B of Fig. 6, taken together, suggest that residues 61-66 of mature human IL-4 constitute an epitope, antibodies against which inhibit the binding of human IL-4 to cellular receptors.

15   This suggestion is strengthened by ELISA studies carried out as described above using polypeptides Nos. 6 and 7 and one of the monoclonal antibodies against polypeptide No. 7. This antibody bound strongly to both of the polypeptides. It also strongly inhibited the binding of ^{125}I-IL-4 to Daudi cells. The only common subsequence in the

20   polypeptides is KDTRC, which corresponds to residues 61-65 of mature human IL-4. It follows that this inhibitory monoclonal antibody must have been directed against this subsequence, and the subsequence must constitute an important epitope.

25   Many modifications and variations of this invention may be made without departing from its spirit and scope, as will become apparent to those skilled in the art. The specific embodiments described herein are offered by way of example only, and the invention is to be limited only by the terms of the appended claims.

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WHAT IS CLAIMED IS:

1. A polypeptide containing from about 5 to about 26 amino acid residues and having an amino acid sequence

5   corresponding to the sequence of amino acid residues 61 to 82 or 104 to 129 of human IL-4, or a subsequence thereof.

2. The polypeptide of claim 1 which is covalently coupled to a carrier molecule.

10

3. The polypeptide of claim 1 which has the amino acid sequence

Lys-Asp-Thr-Arg-Cys,

15

Thr-Ala-Gln-Gln-Phe-His-Arg-His,

Lys-Asp-Thr-Arg-Cys-Leu-Gly-Ala-Thr-Ala-
Gln-Gln-Phe-His-Arg-His-Lys-Gln-Leu-Ile-

20   Arg-Phe or

Ala-Asn-Gln-Ser-Thr-Leu-Glu-Asn-Phe-Leu-
Glu-Arg-Leu-Lys-Thr-Ile-Met-Arg-Glu-Lys-
Tyr-Ser-Lys-Cys-Ser-Ser.

25

4. An antibody which specifically binds to human IL-4 and to a polypeptide containing from about 5 to about 26 amino acid residues and having an amino acid sequence corresponding to the sequence of amino acid residues 61 to 82 or 104 to 129 of human IL-4,

30   or a subsequence thereof, which antibody inhibits the binding of human IL-4 to cellular receptors.

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5. An anti-idiotypic antibody against an antibody which specifically binds to human IL-4 and to a polypeptide containing from about 5 to about 26 amino acid residues and having an amino acid sequence corresponding to the sequence of amino acid residues 61 to 5   82 or 104 to 129 of human IL-4, or a subsequence thereof, which antibody inhibits the binding of human IL-4 to cellular receptors.

6. A method for inhibiting the binding of human IL-4 to cellular receptors comprising contacting human IL-4 with an antibody of 10   claim 4.

7. A method for inhibiting the binding of human IL-4 to cellular receptors comprising contacting cells bearing receptors for human IL-4 with an anti-idiotypic antibody of claim 5. 15

8. A pharmaceutical composition for treating allergies or other conditions mediated by IL-4 comprising a physiologically acceptable carrier and an effective amount of one or more of the antibodies of claim 4. 20

9. A pharmaceutical composition for treating allergies or other conditions mediated by IL-4 comprising a physiologically acceptable carrier and an effective amount of one or more anti-idiotypic antibodies of claim 5. 25

10. A method for making a pharmaceutical composition for treating allergies or other conditions mediated by IL-4 comprising admixing a physiologically acceptable carrier and an effective amount of one or more of the antibodies of either of claims 4 or 5.

Fig. 1

His-Lys-Cys-Asp-Ile-Thr-Leu-Gln-Glu-Ile-

Ile-Lys-Thr-Leu-Asn-Ser-Leu-Thr-Glu-Gln-

Lys-Thr-Leu-Cys-Thr-Glu-Leu-Thr-Val-Thr-

Asp-Ile-Phe-Ala-Ala-Ser-Lys-Asn-Thr-Thr-

Glu-Lys-Glu-Thr-Phe-Cys-Arg-Ala-Ala-Thr-

Val-Leu-Arg-Gln-Phe-Tyr-Ser-His-His-Glu-

Lys-Asp-Thr-Arg-Cys-Leu-Gly-Ala-Thr-Ala-

Gln-Gln-Phe-His-Arg-His-Lys-Gln-Leu-Ile-

Arg-Phe-Leu-Lys-Arg-Leu-Asp-Arg-Asn-Leu-

Trp-Gly-Leu-Ala-Gly-Leu-Asn-Ser-Cys-Pro-

Val-Lys-Glu-Ala-Asn-Gln-Ser-Thr-Leu-Glu-

Asn-Phe-Leu-Glu-Arg-Leu-Lys-Thr-Ile-Met-
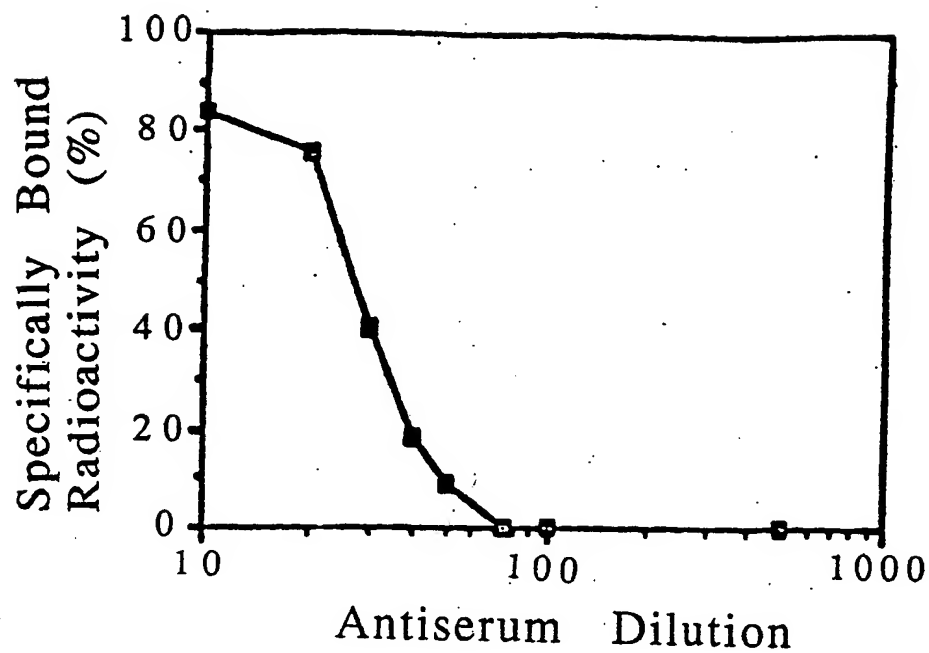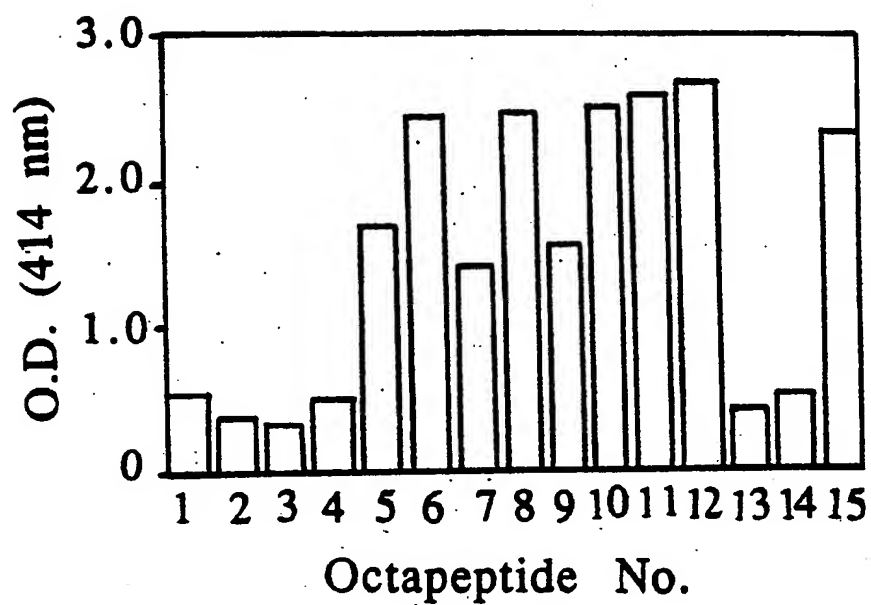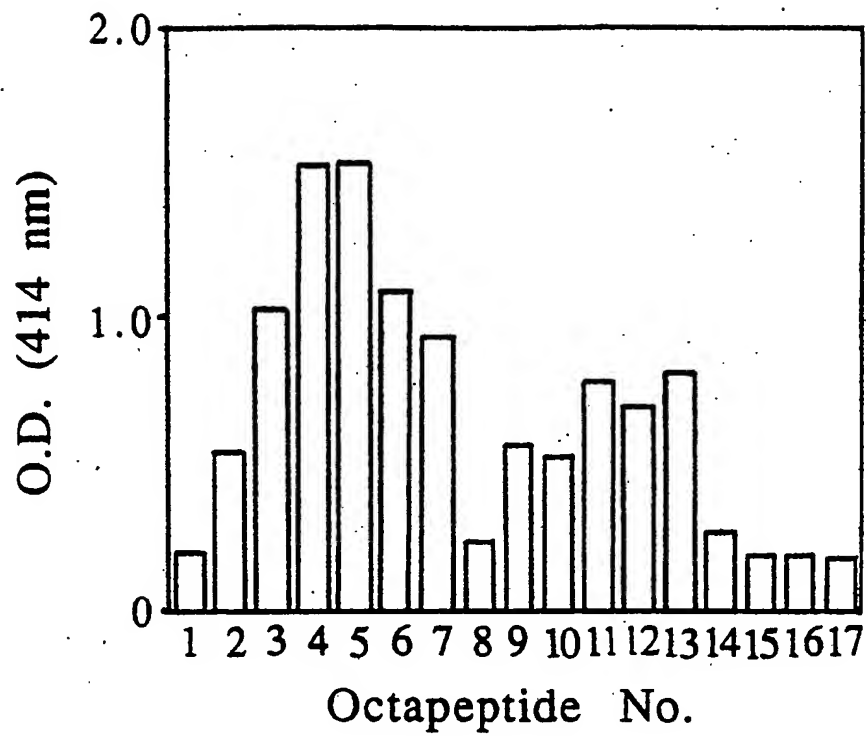
Arg-Glu-Lys-Tyr-Ser-Lys-Cys-Ser-Ser

Fig. 2
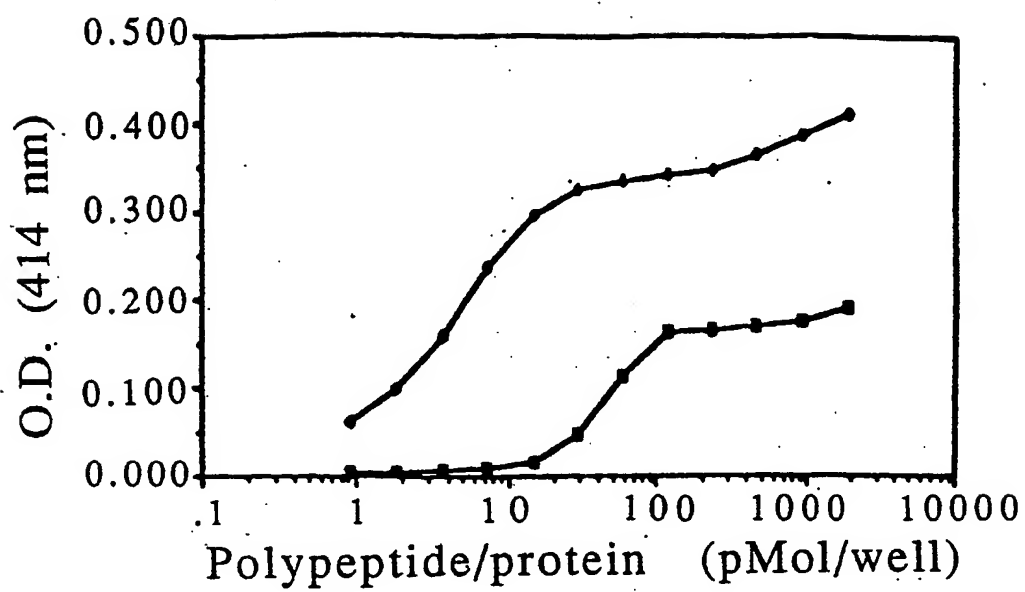
Fig. 3
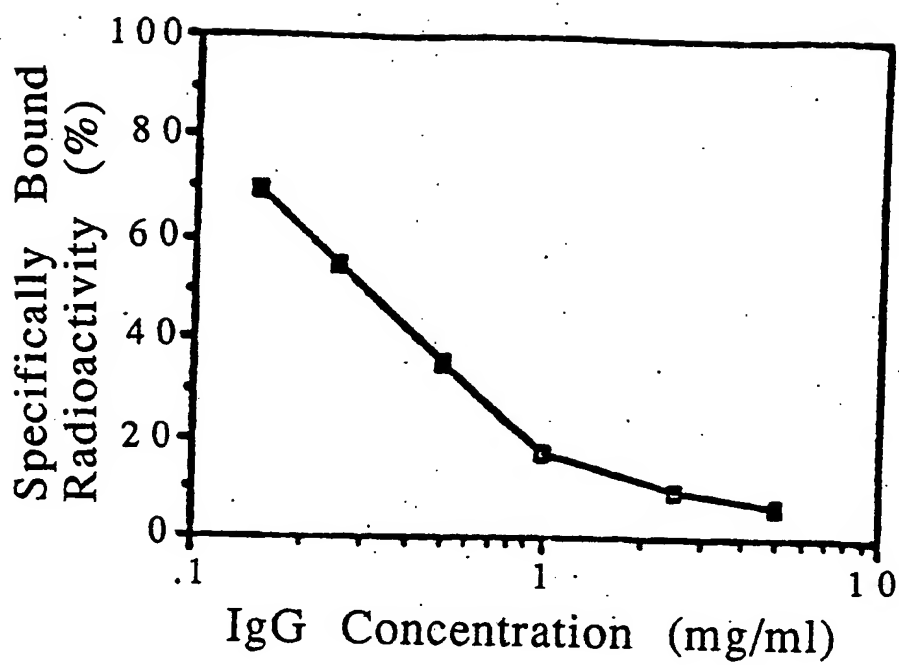
Fig. 4

Fig. 5

Fig. 6A

III. DOCUMENTS CONSIDERED TO BE RELEVANT	(CONTINUED FROM THE SECOND SHEET)	
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